# Supporting Information For:

# Synthesis of acid-labile PEG- and PEG-doxorubicin conjugatenanoparticles via brush-first ROMP

Angela Xiaodi Gao<sup>1</sup>, Longyan Liao<sup>1</sup>, Jeremiah A. Johnson<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139

E-mail: jaj2109@mit.edu

## Table of Contents

S2-S3 Figures referenced in the main text

S4-S6 General Considerations

S7-S10 Preparation of silyl ether crosslinkers

S11-S13 Preparation of acetal crosslinker

S14-S20 Preparation of **DOX-MM** 

S21-S21 BASP formation via brush-first ROMP

S22-S36 NMR spectra of new compounds

S37-S37 MALDI spectrum of **DOX-MM** 

S38-S38 References

## Figures referenced in the main text:

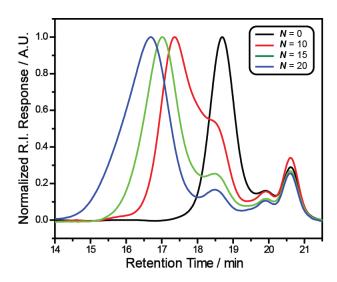
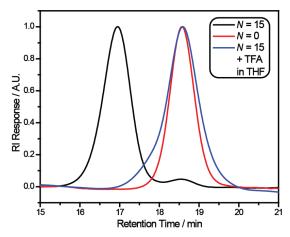
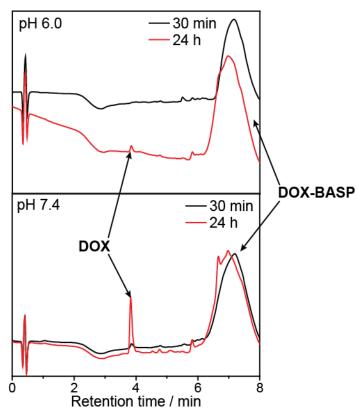


Figure S1. Refractive index GPC traces for silyl ether-based crosslinker 4



*Figure S2*. A. GPC analysis of degraded N = 15 acetal-BASP compared with parent bottlebrush.



*Figure S3*. LC/MS traces of DOX-BASPs after incubation in pH 6.0 (top) and pH 7.4 (bottom) PBS buffers for 30 min and 24 h.

#### **General Considerations**

All reagents and solvents were purchased from Aldrich or VWR and used as supplied unless otherwise noted. Grubbs 3<sup>rd</sup> generation bispyridyl catalyst<sup>1</sup>, **PEG-MM**<sup>2</sup> and **PEG-Alkyne-MM**<sup>3</sup> were prepared according to literature procedures. Degassed dichloromethane (DCM) and tetrahydrofuran (THF) were passed through solvent purification columns prior to use.

Liquid chromatography–mass spectrometry (LC/MS) and preparative HPLC were performed on an Agilent 1260 LC system equipped with a Zorbax SB-C18 rapid resolution HT column and a Zorbax SB-C18 semi-preparative column. Solvent gradients consisted of mixtures of nano-pure water with 0.1% acetic acid (AcOH) and HPLC-grade acetonitrile. Mass spectra were obtained using an Agilent 6130 single quadrupole mass spectrometer.

Dynamic light scattering (DLS) measurements were made at room temperature using a Malvern Zetasizer DLS instrument. Samples were dissolved in nanopure water at a concentration of ~1 mg / mL. A fresh, clean, polystyrene cuvette was washed with compressed air to remove dust. The sample solution was passed through a 0.4 μm Nylon syringe filter directly into the cuvette; the cuvette was capped and placed in the DLS instrument for particle sizing. At least 10 measurements were made per sample and average hydrodynamic diameters were calculated by fitting the DLS correlation function using the zetasizer software.

<sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H-NMR) and <sup>13</sup>C nuclear magnetic resonance (<sup>13</sup>C-NMR) spectra were recorded on Bruker AVANCE-400 NMR spectrometer or INOVA 500 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the CHCl<sub>3</sub> singlet at 7.24 ppm, DMSO at 2.50 ppm, MeOH at 4.87 ppm, or CH<sub>2</sub>Cl<sub>2</sub> at 5.32 ppm. <sup>13</sup>C-NMR spectra were referenced to the center peaks of the CDCl<sub>3</sub> triplet at 77.23 ppm, DMSO septet at 39.51 ppm,

MeOH septet at 49.150, or  $CD_2Cl_2$  quintet at 54.0 ppm. Chemical shifts are expressed in parts per million (ppm), and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet) and br (broad). Coupling constants J are reported in Hertz (Hz). MestReNova NMR 7.0.1 software was used to analyze the NMR spectra.

Gel permeation chromatography (GPC) measurements were performed on an Agilent 1260 LC system with two Shodex KD-806M GPC columns in series at 60 °C and a flow rate of 1 mL / min. *N*,*N*-Dimethylformamide (DMF) with 0.02 M LiBr was used as the eluent. A T-rEX refractive index detector (Wyatt) and a DAWN EOS 18-angle laser light scattering (MALLS) detector (Wyatt) were used for polymer analysis.

High-resolution mass spectrometry (HRMS) was obtained using a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS).

TEM images were obtained at the MIT Center for Materials Science and Engineering on a FEI\_Tecnai Digital TEM. The samples were prepared as follows: 5.0 μL of a 1.0 mg/mL solution of BASP polymer was deposited via pipet on top of a carbon film-coated 200-mesh copper grid (purchased from Electron Microscopy Sciences) placed on a piece of parafilm carbon-coated side up. After 2 min, the excess of the solution was quickly wicked away by a piece of filter paper. The samples were then negatively stained with 2 wt% uranyl acetate aqueous solution. After 1 min, the excess staining solution was quickly wicked away by a piece of filter paper and the samples were left to dry under ambient conditions and then was ready for TEM imaging.

*Cell Culture Studies:* HeLa cells (ATCC) were maintained in RPMI-1640 media supplemented with 0.01 mg/mL recombinant human insulin (Gibco), 20% fetal bovine serum,

and penicillin/streptomycin in a 5% CO<sub>2</sub> humidified atmosphere (37 °C). Assays were performed on cells passaged 24 h prior. Dose-response curves were fit using a four-parameter logistic regression analysis in Origin 8.5.1 software. BASP drug conjugates were reconstituted in ultrapure water (18 M $\Omega$ ) and stored at 4 °C in dark prior to use. Viability was assessed by CellTiter-Glo assay (Promega) following 72 h total incubation time with ABASPs-spiked Opti-MEM reduced serum media.

#### **Synthetic Procedures**

#### Synthesis of Silyl Ether Crosslinkers 1-4

Compound C1. *Cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (0.625 g, 3.81 mmol), 3-aminobenzyl alcohol (0.516 g, 4.18 mmol), N,N-diisopropylethylamine (0.730 mL, 4.18 mmol), and toluene (14 mL) were added to a microwave vial. This reaction mixture was heated and stirred in a microwave reactor at 170 °C for 30 min. The crude product was purified via silica gel chromatography (0%-50% EtOAc/hexanes) to give C1 (0.810 g, 79% yield) as a pure white solid.  $^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.42 (t, J= 7.8 Hz, 1H), 7.34 (d, J = 7.9 Hz, 1H), 7.23 (s, 1H), 7.14 (d, J = 7.8 Hz, 1H), 6.32 (t, J = 1.8 Hz, 2H), 4.67 (s, 2H), 3.37-3.36 (m, 2H), 2.82 (s, 2H), 2.08 (s, 1H), 1.59 (dt, J = 9.9, 1.4 Hz, 1H), 1.47 (d, J = 9.9 Hz, 1H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  177.31, 142.65, 138.19, 132.17, 129.52, 127.24, 125.68, 124.86, 64.73, 48.09, 46.01, 43.18. HRMS: calcd. for C<sub>16</sub>H<sub>15</sub>NO<sub>3</sub> [M+NH<sub>4</sub>]<sup>+</sup>, 287.1390; found, 287.1379.

**Compound C2**. *Cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (1.863 g, 11.4 mmol), ethanolamine (0.891 mL, 14.8 mmol), *N*,*N*-diisopropylethylamine (0.198 mL, 1.14 mmol), and

toluene (14 mL) were added to a microwave vial. This reaction mixture was heated and stirred in a microwave reactor at 175 °C for 30 min. The reaction was then diluted with dichloromethane (100 mL) and washed with water (3 x 100 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and concentrated via rotary evaporation to give **C2** (1.54 g, 65% yield) as a pure white solid.  $^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.26-6.25 (m, 2H), 3.74-3.71 (m, 2H), 3.67-3.64 (m, 2H), 3.24 (s, 2H), 2.68 (s, 2H), 2.34 (s, 1H), 1.48 (dt, J = 9.9, 1.4 Hz, 1H), 1.31 (d, J = 9.9 Hz, 1H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  178.92, 138.00, 77.23, 60.47, 48.06, 45.44, 42.95, 41.47. HRMS: calcd. for C<sub>11</sub>H<sub>13</sub>NO<sub>3</sub> [M+H]<sup>+</sup>, 208.0968; found, 208.0958.

Crosslinker 1. Compound C2 (0.300 g, 1.45 mmol) and *N*,*N*-diisopropylethylamine (0.419 mL, 2.41 mmol) were dissolved in 3 mL of dichloromethane. To this solution, dichlorodiisopropylsilane (0.065 mL, 0.362 mmol) was added dropwise at room temperature. The reaction was stirred overnight and then concentrated via rotary evaporation. The crude product was purified via silica gel chromatography (0%-30% EtOAc/hexanes) to give crosslinker 1 (0.112 g, 59% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.19-6.18 (m, 4H), 3.78 (t, J = 5.7, 4H), 3.56 (t, J = 5.7, 4H), 3.16 (s, 4H), 2.58 (s, 4H), 1.38 (dt, J = 9.8, 1.4 Hz, 2H), 1.29 (d, J = 9.8 Hz, 2H), 0.87-0.83 (m, 14H). <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  178.00, 137.84, 59.04, 47.82, 45.27, 42.86, 40.68, 17.10, 11.85. LC/MS *m/z*: calcd. for C<sub>28</sub>H<sub>38</sub>N<sub>2</sub>O<sub>6</sub>Si [M+Na]<sup>+</sup>, 549.2; found, 549.3.

**Crosslinker 2.** Compound **C2** (0.300 g, 1.45 mmol) and *N*,*N*-diisopropylethylamine (0.419 mL, 2.41 mmol) were dissolved in 3 mL of dichloromethane. To this solution, dichlorodiphenylsilane (0.076 mL, 0.362 mmol) was added dropwise at room temperature. The reaction was stirred overnight and then concentrated via rotary evaporation. The crude product was purified via silica gel chromatography (0%-30% EtOAc/hexanes) to give crosslinker **1** (0.075 g, 35% yield).  $^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.54-7.52 (m, 4H), 7.41-7.37 (m, 2H), 7.34-7.30 (m, 4H), 6.24-6.23 (m, 4H), 3.91 (t, J = 5.5 Hz, 4H), 3.67 (t, J = 5.5, 4H), 3.21 (s, 4H), 2.60 (s, 4H), 1.33 (s, 4H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>) δ 178.21, 137.96, 135.02, 131.67, 130.74, 128.06, 59.52, 47.99, 45.40, 42.98, 40.68. LC/MS m/z: calcd. for C<sub>34</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>Si [M+Na]<sup>+</sup>, 617.2; found, 617.2. \*Minor impurity and solvent peaks:  $^{1}$ H NMR (400 MHz, DMSO- $^{2}$ d<sub>6</sub>) δ 4.09 (q, J = 7.1 Hz, 0.08H), 3.77-3.74 (m, 0.21H), 3.26 (s, 0.18H), 2.69 (s, 0.18H), 2.01 (s, 0.08H), 1.23 (t, J = 7.1, 0.14H).

**Crosslinker 3.** Compound **C3** (0.122 g, 0.451 mmol) and *N,N*-diisopropylethylamine (0.110 mL, 0.632 mmol) were dissolved in 3 mL of dichloromethane. To this solution, dichlorodiisopropylsilane (0.039 mL, 0.216 mmol) was added dropwise at room temperature.

The reaction was stirred overnight and then concentrated via rotary evaporation. The crude product was purified via silica gel chromatography (0%-50% EtOAc/hexanes) to give crosslinker **3** (0.106 g, 76% yield).  $^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.38 (d, J = 8.4, 4H), 7.20-7.18 (m, 4H), 6.33-6.32 (m, 4H), 4.82 (s, 4H), 3.38 (s, 4H), 2.83 (s, 4H), 1.59 (dt, J = 9.9, 1.3 Hz, 2H), 1.47 (d, J = 9.9 Hz, 2H), 1.11-1.07 (m, 14H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  177.30, 141.76, 138.20, 130.71, 126.75, 126.40, 64.26, 48.06, 46.01, 43.17, 17.60, 12.37. LC/MS m/z: calcd. for  $C_{34}H_{34}N_2O_6Si$  [M+Na]<sup>+</sup>, 617.2; found, 617.2.

Crosslinker 4. Compound C1 (0.120 g, 0.405 mmol) and *N*,*N*-diisopropylethylamine (0.100 mL, 0.567 mmol) were dissolved in 3 mL of dichloromethane. To this solution, dichlorodiphenylsilane (0.045 mL, 0.212 mmol) was added dropwise at room temperature. The reaction was stirred overnight and then concentrated via rotary evaporation. The crude product was purified via silica gel chromatography (0%-50% EtOAc/hexanes) to give crosslinker 4 (0.112 g, 73% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.71-7.68 (m, 4H), 7.45-7.32 (m, 10H), 7.25-7.22 (m, 2H), 7.17-7.11 (m, 2H), 6.33-6.32 (m, 4H), 4.85 (s, 4H), 3.37 (s, 4H), 2.82 (s, 4H), 1.57-1.49 (m, 2H), 1.44-1.30 (m, 2H). <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>) δ 177.16, 141.82, 138.16, 135.14, 132.05, 132.01, 130.75, 129.26, 128.19, 126.83, 125.30, 234.58, 64.55, 48.02, 45.98, 43.13. LC/MS *m/z*: calcd. for C<sub>44</sub>H<sub>38</sub>N<sub>2</sub>O<sub>6</sub>Si [M+H]<sup>+</sup>, 720.3; found, 720.2.

\* Minor impurity peaks:  $^{1}$ H NMR (400 MHz, DMSO- $d_{6}$ )  $\delta$  4.68 (s, 0.24H), 1.97 (s, 0.27H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  142.65, 129.48, 127.18, 125.65, 124.83.

#### Synthesis of Acetal Crosslinker 5

Compound A1. To a 20 mL microwave vial equipped with a magnetic stir bar was added a solution of *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (1.175 g, 7.15 mmol), 4-aminophenol (1.013 g, 9.28 mmol), and *N*,*N*-diisopropylethylamine (1.62 mL, 9.28 mmol) in toluene (17 mL). The vial was capped and the solution was placed in the microwave at 140°C for 4 hours. The reaction was then concentrated via rotary evaporator, re-dissolved in hot methanol (20 mL), and filtered immediately. The resulting filtrate was cooled to 0°C to give off-white crystals. The crystals were filtered and recrystallized twice in methanol to give A1 as white crystals (1.133 g, 63% yield). <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  9.73 (s, 1H), 7.04-7.00 (m, 2H), 6.84-6.81 (m, 2H), 6.36-6.35 (m, 2H), 3.19-3.18 (m, 2H), 2.80 (s, 2H), 1.47-1.44 (m, 1H), 1.38 (d, J = 9.8 Hz, 1H). <sup>13</sup>C-NMR (400 MHz, DMSO- $d_6$ )  $\delta$  177.15, 157.34, 137.76, 123.35, 115.39, 47.34, 44.92, 42.62. HRMS: calcd. for C<sub>15</sub>H<sub>12</sub>NO<sub>3</sub> [M+H]<sup>+</sup>, 256.0968; found, 256.0965.

\* Minor solvent peak:  ${}^{1}$ H NMR (400 MHz, DMSO- $d_{6}$ )  $\delta$  3.34 (s, 0.13H).

Compound A2. A solution of A1 (0.616 g, 2.41 mmol) and pyridinium *p*-toluenesulfonate (0.061 g, 0.241 mmol) in DCM (60 mL) was added to a dried, 150 mL round-bottom flask. The solution was cooled down to 0°C in an ice bath and 2-chloroethyl vinyl ether (1.22 mL, 12.0 mmol) was added dropwise. The reaction was stirred for 12 h under nitrogen atmosphere, and then diluted with DCM (50 mL), transferred to a separatory funnel, and washed with saturated sodium bicarbonate (100 mL), water (100 mL), and brine (100 mL). The organic solution was dried over sodium sulfate, filtered, and concentrated via rotary evaporation. The crude product was purified via silica gel chromatography (0%-30% EtOAc/hexanes) to give A2 (0.804 g, 92% yield\*). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.16-7.12 (m, 2H), 7.08-7.04 (m, 2H), 6.30 (t, J =1.8, 2H), 5.45 (q, J = 5.4, 1H), 3.92-3.86 (m, 1H), 3.75-3.69 (m, 1H), 3.58-3.54 (m, 2H), 3.35 (s, 2H), 2.79 (s, 2H), 1.57 (d, J = 9.9, 1H), 1.42 (d, J = 9.9, 1H). <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  177.34, 156.64, 138.07, 127.72, 125.81, 117.65, 99.49, 65.20, 47.91, 45.89, 43.06, 43.01, 19.87. LC/MS *m/z*: calcd. for C<sub>19</sub>H<sub>20</sub>CINO<sub>4</sub> [M+H]<sup>+</sup>, 362.1; found, 362.1.

<sup>\*</sup> Minor solvent peaks:  $^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.25 (s, 0.17H), 4.08 (q, J = 7.1, 0.29H), 2.00 (s, 0.45H), 1.21 (t, J = 7.2, 0.51H).

Crosslinker 5. A solution of A2, A1, Na<sub>2</sub>CO<sub>3</sub>, and 15-crown-5 in DMF (15 mL) were added to a dried, 100 mL round-bottom flask. The solution was stirred at 75°C for 48 h under nitrogen. The reaction was then diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub>, water, and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated via rotary evaporation. The crude product was purified via silica gel chromatography (0%-50% EtOAc/hexanes) to give crosslinker 5 (0.241 g, 23% yield\*). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ7.16-7.08 (m, 6H), 6.92-6.90 (m, 2H), 6.32 (m, 4H), 5.51 (q, J = 5.3 Hz, 1H), 4.10-4.08 (m, 2H), 4.02-3.97 (m, 1H), 3.88-3.83 (m, 1H), 3.38-3.37 (m, 4H), 2.81 (d, J = 5.7, 4H), 1.59 (dqt, J = 9.9, 1.5 Hz, 2H), 1.55 (d, J = 6.8 Hz, 3H), 1.45 (d, J = 9.9 Hz, 2H). <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>) 177.51, 177.48, 158.84, 156.90, 138.20, 138.18, 127.81, 127.79, 125.79, 124.93, 117.82, 115.37, 99.49, 67.62, 63.30, 48.04, 48.01, 46.00, 45.99, 43.19, 43.18, 20.04. HRMS: calcd. for C<sub>34</sub>H<sub>42</sub>N<sub>2</sub>O<sub>7</sub> [M+H]<sup>+</sup>, 581.2282; found, 581.2269.

\* Minor solvent peak: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.52 (s, 1.25H).

#### Synthesis of DOX-MM

Compound B1. To a dried 200 mL round bottom flask was added 6-bromohexanoic acid (4.00 g, 20.5 mmol), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (4.30 g, 22.5 mmol), and 4-(dimethylamino)pyridine (0.250 g, 2.05 mmol) in 120 mL of anhydrous dichloromethane. The solution was stirred for 5 minutes, after which 4-hydroxybenzaldehyde (2.50 g, 20.5 mmol) was added. The reaction was stirred at room temperature overnight. The reaction was then transferred to a separatory funnel and washed with water (2 x 150 mL) and brine (150 mL). The organic layer was dried over magnesium sulfate, which was removed by filtration. The solution was concentrated and run through a silica plug in ethyl acetate. The fractions were collected and the solvent was removed by rotary evaporation and dried overnight to give compound B1 (4.5 g, 74% yield\*).  $^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>) 9.96 (s, 1H), 7.91-7.88 (m, 2H), 7.27-7.23 (m, 2H), 3.42 (t, J = 6.7, 2H), 2.60 (t, J = 7.4, 2H), 1.94-1.73 (m, 4H), 1.60-1.52 (m, 2H).  $\delta$   $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  191.11, 171.43, 155.59, 134.19, 131.42, 122.55, 44.89, 34.36, 32.35, 26.49, 24.25. HRMS: calcd. for  $C_{13}$ H<sub>15</sub>BrO<sub>3</sub> [M+NH<sub>4</sub>]\*, 316.0543; found, 316.0532.

\* Minor impurity and solvent peaks:  ${}^{1}$ H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  4.09 (q, J = 7.1, 0.13H), 3.55 (t, J = 6.6, 0.46H), 2.14 (s, 0.35H), 2.02 (s, 0.20H), 1.23 (t, J = 7.1, 0.21 H)

Compound B2. Sodium borohydride (0.760 g, 20.1 mmol) was added to compound B1 (4.00 g, 13.4 mmol) in anhydrous methanol (30 mL) at 0 °C under nitrogen. The reaction was stirred for 5 min then quenched with the addition of water. 1M HCl was added to neutralize the reaction mixture until pH = 7. The mixture was extracted with dichloromethane (3 x 50 mL), and the organic layers were combined and dried with anhydrous magnesium sulfate, which was removed by filtration. The solution was concentrated and silica gel chromatography was performed using a gradient of 10% to 80% ethyl acetate in hexanes. The fractions containing product were collected, and the solvent was removed by rotary evaporation and dried overnight to give compound B2 (2.7 g, yield 67%\*). ¹H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.37-7.33 (m, 2H), 7.06-7.03 (m, 2H), 4.65 (s, 2H), 3.42 (t, J = 6.7 Hz, 2H), 2.56 (t, J = 7.4, 2H), 1.94-1.87 (m, 2H), 1.87-1.80 (m, 3H), 1.59-1.52 (m, 2H). ¹³C-NMR (400 MHz, CDCl<sub>3</sub>) δ 172.20, 150.25, 138.67, 128.26, 121.84, 64.92, 34.31, 33.64, 32.54, 27.79, 24.24. HRMS: calcd. for C<sub>13</sub>H<sub>17</sub>BrO<sub>3</sub> [M+NH<sub>4</sub>]<sup>+</sup>, 318.0699; found, 318.0684.

\* Minor impurity and solvent peaks: 1H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.27 (s, 0.04H), 4.10 (q, J = 7.1, 0.11H), 3.62 (t, J = 6.5 Hz, 0.19H), 3.55 (t, J = 6.6 Hz, 0.18H), 2.14 (s, 0.04H), 2.02 (s, 0.16H), 1.23 (t, J = 7.1 Hz, 0.19H), 0.05 (s, 0.10H).

Compound B3. DMF (20 mL) was added to compound B2 (2.0 g, 6.64 mmol) and sodium azide (0.650 g, 10 mmol) in a flask, which was heated to 60 °C and stirred for 24 hours. The reaction was diluted in ethyl acetate (100 mL), and washed with water (2 x 75 mL) and brine (75 mL). The organic layer was dried with anhydrous magnesium sulfate, which was then filtered. The solution was concentrated and silica gel chromatography was performed using a gradient of 10% to 70% ethyl acetate in hexanes. The fractions containing product were collected, and the solvent was removed by rotary evaporation and dried overnight to give compound B3 (0.716 g, yield 41%). H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.36-7.33 (m, 2H), 7.06-7.02 (m, 2H), 4.65 (s, 2H), 3.28 (t, J = 6.5 Hz, 2H), 2.56 (t, J = 7.4 Hz, 2H), 1.80-1.73 (m, 3H), 1.68-1.61 (m, 2H), 1.52-1.44 (m, 2H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  172.19, 150.24, 138.68, 128.25, 121.82, 64.90, 51.40, 34.32, 28.75, 26.40, 24.59. HRMS: calcd. for C<sub>13</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub> [M+NH<sub>4</sub>]<sup>+</sup>, 281.1608; found, 281.1594.

\* Minor impurity and solvent peaks: 1H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.61 (t, J = 6.5 Hz, 0.16H), 2.14 (s, 0.11H), 1.39-1.36 (m, 0.28H), 0.05 (s, 0.08H).

**Compound B4.** A solution of compound **B3** (0.179 g, 0.680 mmol) and triethylamine (0.134 mL, 0.954 mmol) in tetrahydrofuran (3 mL) was added dropwise to a flask of 4-nitrophenyl chloroformate (0.257 g, 1.28 mmol) in tetrahydrofuran (8 mL) at 0 °C under nitrogen. The icebath was removed and the reaction was left to stir for one hour. The mixture was concentrated via rotary evaporator and purified by silica gel chromatography from 0% to 50% ethyl acetate in hexanes. The fractions containing product was concentrated on a rotary evaporator and dried on vacuum overnight to give **B4** (0.2138 g, yield 73%\*). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.27-8.23 (m, 2H), 7.46-7.42 (m, 2H), 7.38-7.34 (m, 2H), 7.13-7.09 (m, 2H), 5.26 (s, 2H), 3.29 (t, J = 6.8 Hz, 2H), 2.58 (t, J = 7.4 Hz, 2H), 1.78 (p, J = 7.8 Hz, 2H), 1.65-1.62 (m, 2H), 1.53-1.46 (m, 2H). <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>) δ 171.97, 155.68, 152.62, 151.39, 145.64, 131.96, 130.26, 125.51, 122.20, 121.98, 70.47, 51.40, 34.32, 28.76, 26.41, 24.57. HRMS: calcd. for C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O<sub>7</sub> [M+NH<sub>4</sub>]\*, 446.1670; found, 446.1661.

\* Minor impurity and solvent peaks: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.03-8.00 (m, 0.16H), 4.49-4.47 (m, 0.17H), 3.88-3.86 (m, 0.17H), 2.15 (s, 0.10H), 0.05 (s, 0.22H). <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>) δ 129.87, 128.54.

**Synthesis of compound DOX-N**<sub>3</sub>. Doxorubicin hydrochloride (0.304 g, 0.524 mmol) was dissolved in dimethlyformamide (7 mL). N,N-Diisopropylethylamine (0.912 mL, 0.524 mmol) and **B4** (0.2138 g, 0.499 mmol) were added. The solution was stirred at room temperature overnight. The mixture was then diluted with ethyl acetate (75 mL) and washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, which was removed by filtration. The solution was concentrated and silica gel chromatography was performed using a gradient of 0% to 10% methanol in dichloromethane. The fractions containing product were collected, and the solvent was removed by rotary evaporation and dried overnight to give **DOX-N**<sub>3</sub> (0.337 g, yield 81%\*). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  13.95 (s, 1H), 13.21 (s, 1H), 8.01 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 8.36 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.30

(d, J= 8.1 Hz, 2H), 7.00 (d, J= 8.1 Hz, 2H), 5.48 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H), 5.26 (bs, 1H), 5.12 (d, J= 3.6 Hz, 1H),8.4, 1H), 4.99 (s, 2H), 4.74 (s, 2H), 4.53 (bs, 1H), 4.14-4.09 (m, 1H), 4.06 (s, 3H), 3.83 (bs, 1H), 3.64 (s, 1H), 3.29-3.22 (m, 3H), 3.00-2.86 (m, 2H), 2.53 (t, J = 7.4, 2H), 2.31 (d, J = 14.7 Hz, 1H), 2.15 (dd, J = 7.8, 4.0 Hz, 1H), 1.86 (dd, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, 3H), 1.63 (p, J = 13.4, 5.2 Hz, 1H), 1.79-1.70 (m, J= 7.3, 2H), 1.50-1.42 (m, 2H), 1.26 (d, J = 6.6 Hz, 3H).  $^{13}$ C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  214.05, 187.28, 186.89, 172.02, 161.26, 156.39, 155.84, 155.61, 150.65, 135.98, 135.69, 135.98, 135.69, 134.17, 133.79, 129.59, 121.82, 121.07, 120.05, 118.67, 111.79, 111.62, 100.93, 77.43, 76.83, 69.88, 69.74, 67.46, 66.29, 65.76, 56.88, 51.40, 47.22, 35.83, 34.31, 34.21, 30.36, 29.48, 28.75, 26.40, 24.57, 17.03. HRMS: calcd. for C<sub>41</sub>H<sub>44</sub>N<sub>4</sub>O<sub>15</sub> [M+NH<sub>4</sub>]<sup>+</sup>, 850.3141; found, 850.3150.

\*Observed silicone grease: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.048 (s, 0.53H)

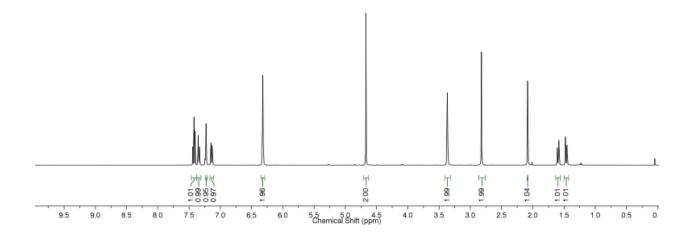
Synthesis of DOX-MM To a solution of DOX-N<sub>3</sub> (32.3 mg, 0.039 mmol) and PEG-Alkyne-MM<sup>3</sup> (120 mg, 0.036 mmol) in 5 ml DCM was added copper(I) acetate (13.2 mg, 0.107 mmol) under N<sub>2</sub> atmosphere. The reaction was allowed to stir at room temperature and monitored by LC-MS until complete consumption of **PEG-Alkyne-MM**. Then the solvent was removed under vacuum and the residue was purified by preparatory HPLC using a linear gradient from 5% MeCN: 95% H<sub>2</sub>O to 95% MeCN: 5% H<sub>2</sub>O over 20 minutes. The desired **DOX-MM** was obtained as a red powder (35 mg, 23% yield). <sup>1</sup>H NMR and MALDI are provided at the end of the chapter.

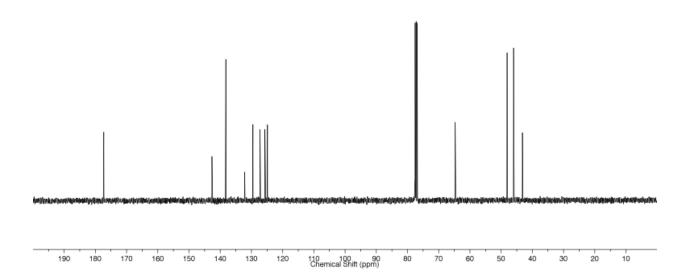
#### General Procedure for BASP Synthesis

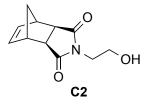
Synthesis of silyl-ether and acetal-core BASPs. PEG-MM was added to a 4 mL vial containing a stir bar. THF was added to the vial with PEG-MM followed with a freshly prepared solution of Gruubs  $3^{rd}$  generation bis-pyridyl catalyst in THF (0.02 g catalyst / 1 mL THF, amount added to give desired PEG-MM:catalyst = 10) such that the total concentration of PEG-MM was 0.1 M. After 10 minutes of stirring at 25°C, aliquots of the polymerization mixture was transferred to 4 x 4 mL empty vials. A stock solution of crosslinker 5 in THF (0.1 M) was dispensed into the vials in batches of 5 equivalents until the desired N equivalents were added. The resulting mixtures were stirred at 25 °C for 1 hr, at which point 1 drop of ethyl vinyl ether was added to quench the polymerization.

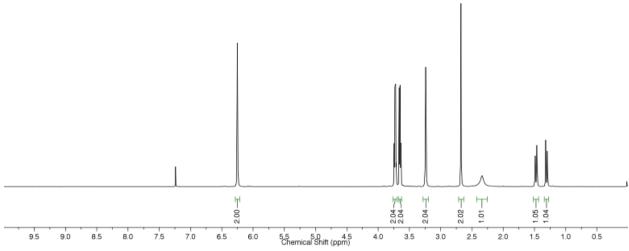
Synthesis of DOX-BASP. DOX-MM (30 mg) was added to a 4 mL vial containing a stir bar. THF (45.1  $\mu$ L) was added to the vial with DOX-MM followed by a freshly prepared solution of Gruubs 3<sup>rd</sup> generation bis-pyridyl catalyst in THF (0.02 g/mL, 25.8  $\mu$ L) to give desired DOX-MM:catalyst = 10. Note that the total concentration of MM DOX-MM was 0.1 M. The solution was allowed to stir for 10 minutes at 25 °C; meanwhile, a stock solution of crosslinker 5 in THF (0.1M) was made. The stock solution of crosslinker 5 was slowly dispensed into the vial in batches of 5 equivalents (31.9  $\mu$ L) until N = 15 equivalents (95.7  $\mu$ L total) were added. The resulting mixtures were stirred at 25 °C for 1 hr, at which point 1 drop of ethyl vinyl ether was added to quench the polymerization.

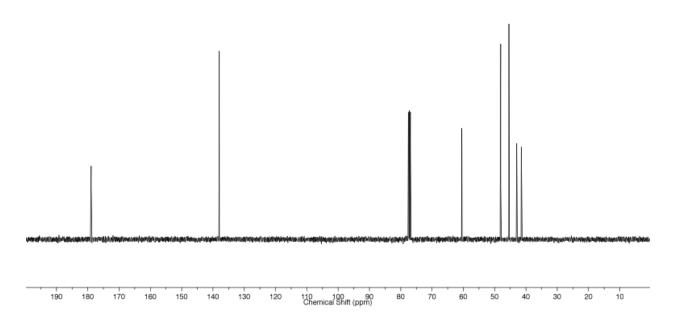
## Spectral Data



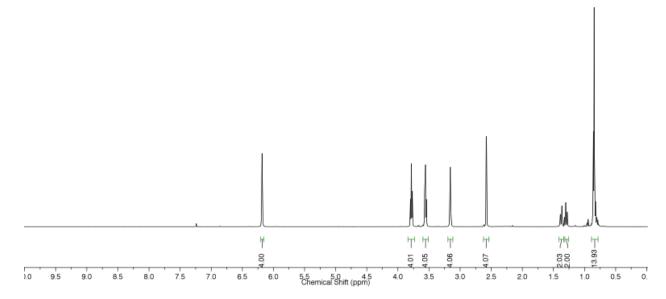


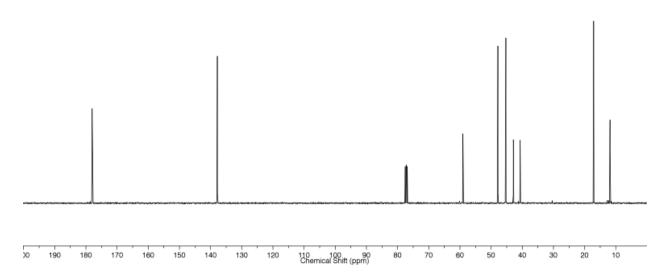




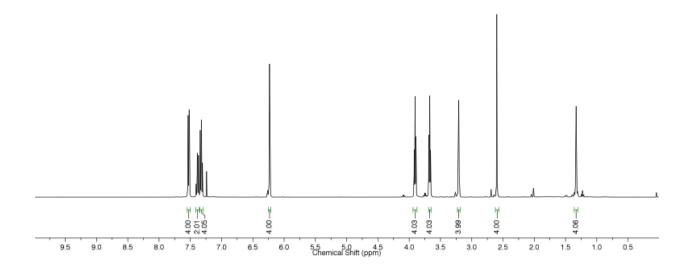


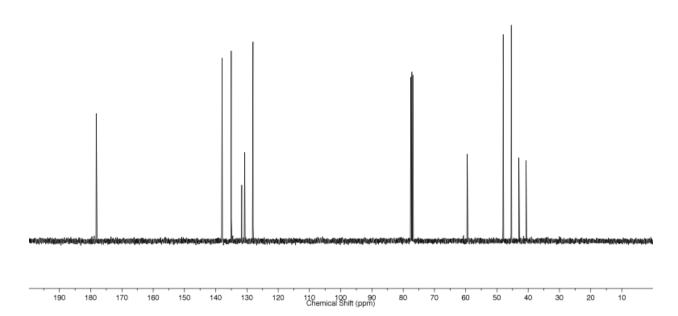
Crosslinker 1



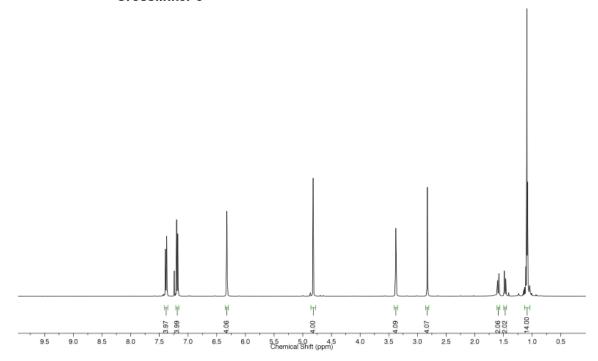


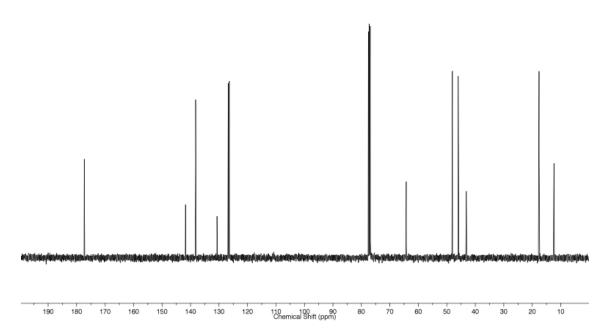
Crosslinker 2



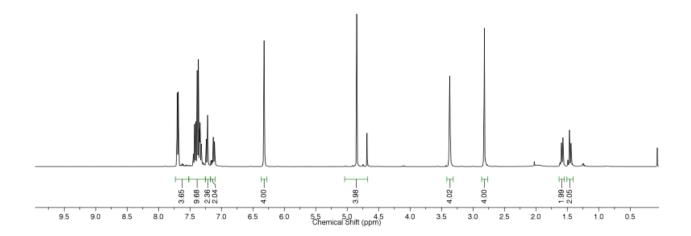


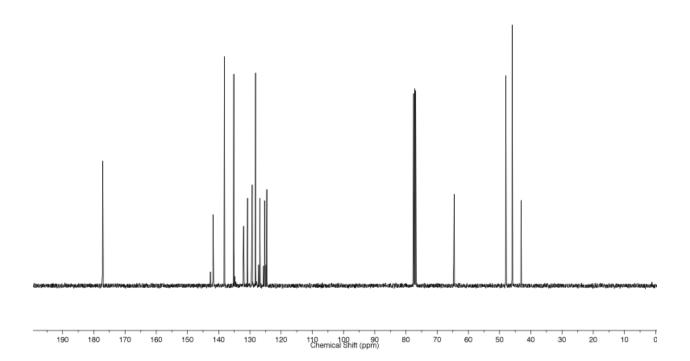
## Crosslinker 3



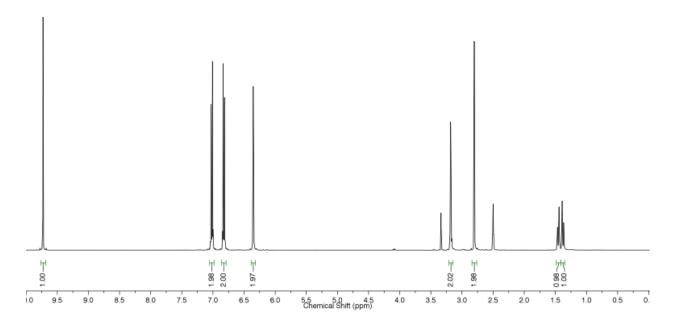


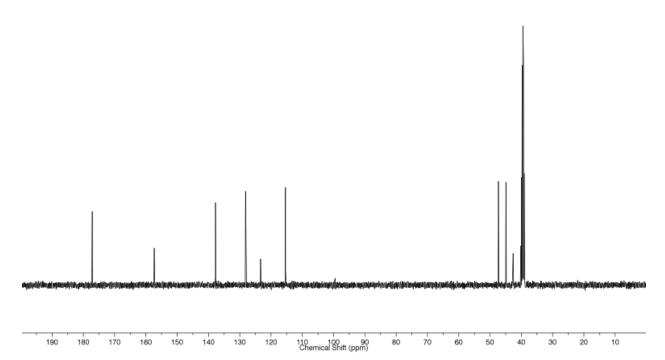
Crosslinker 4

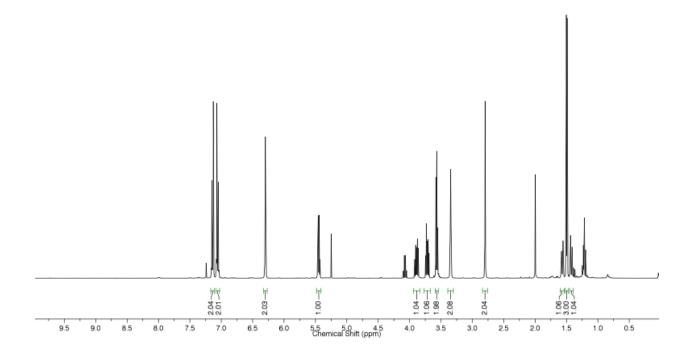


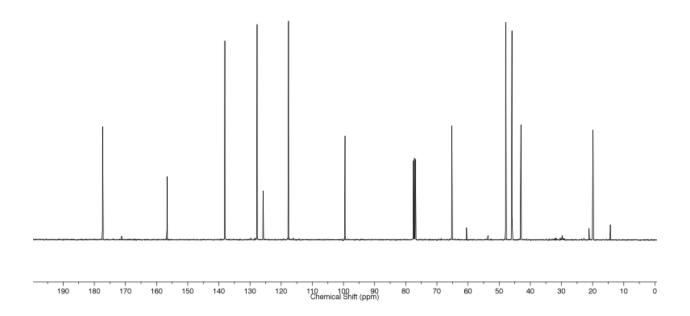


**A**1

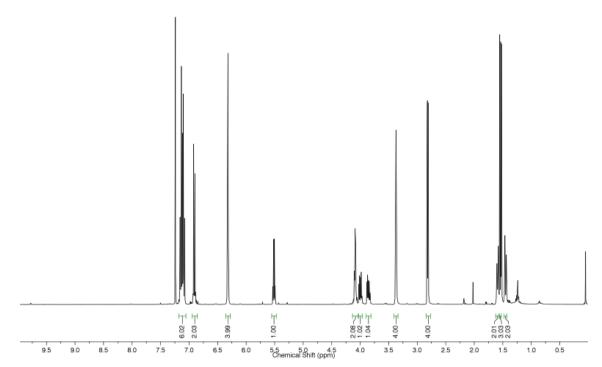


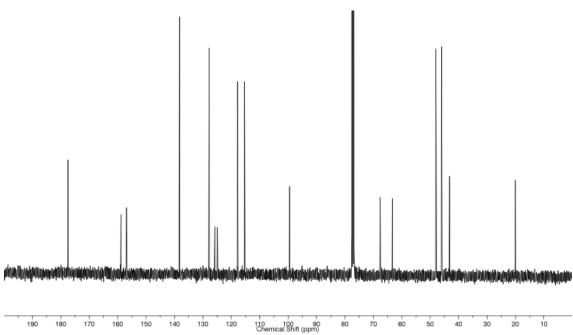


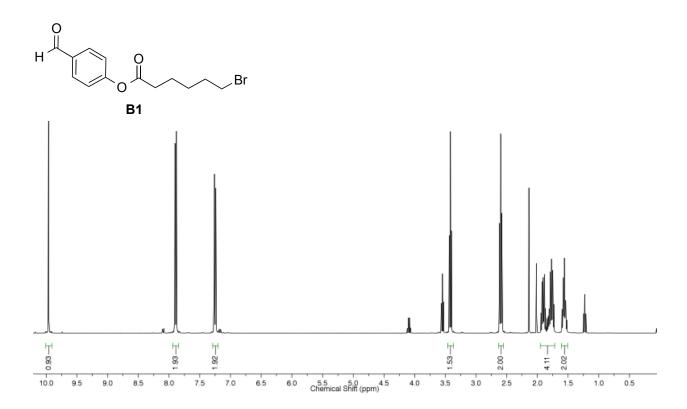


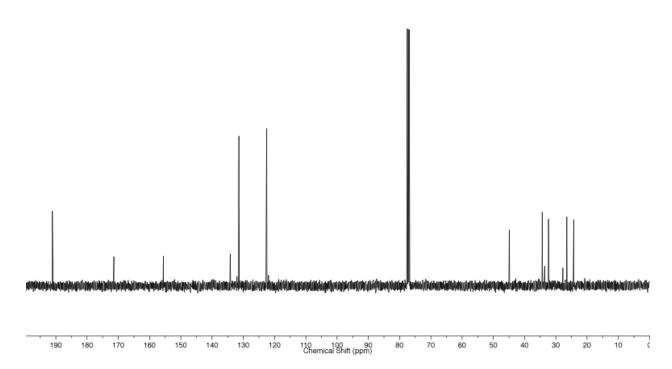


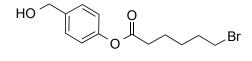
## crosslinker 5



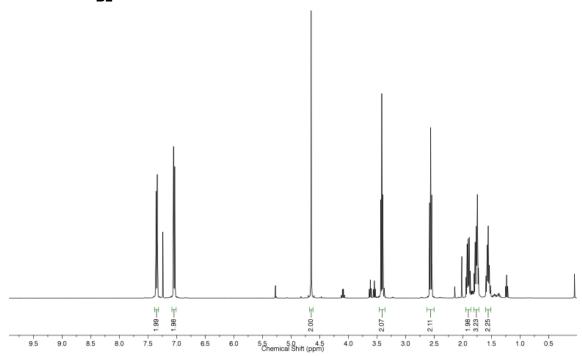


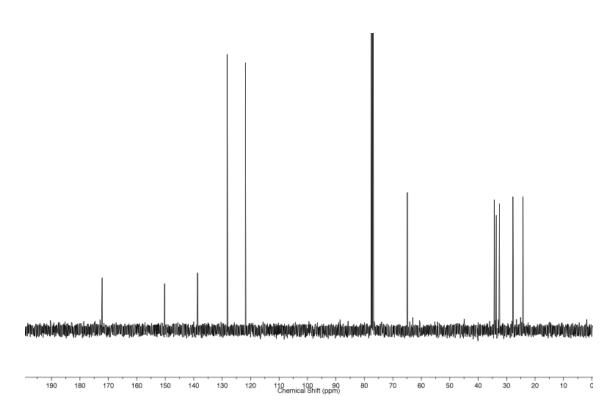


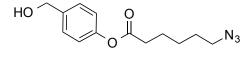




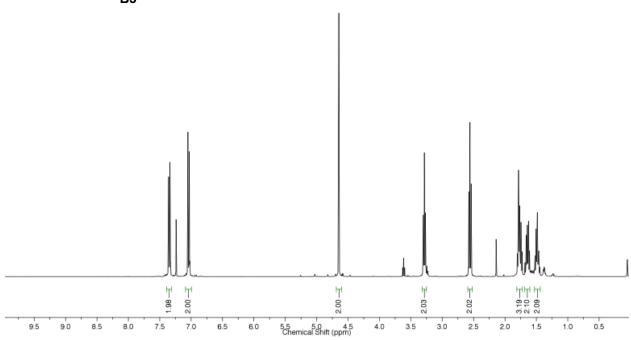
В2

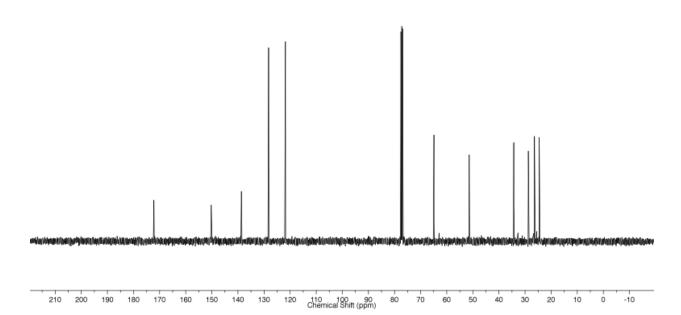


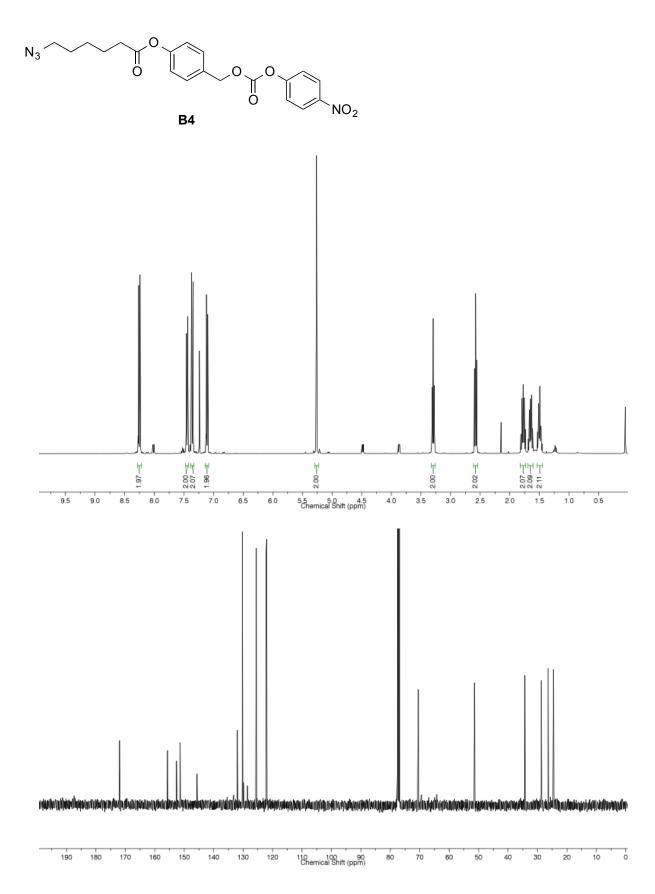


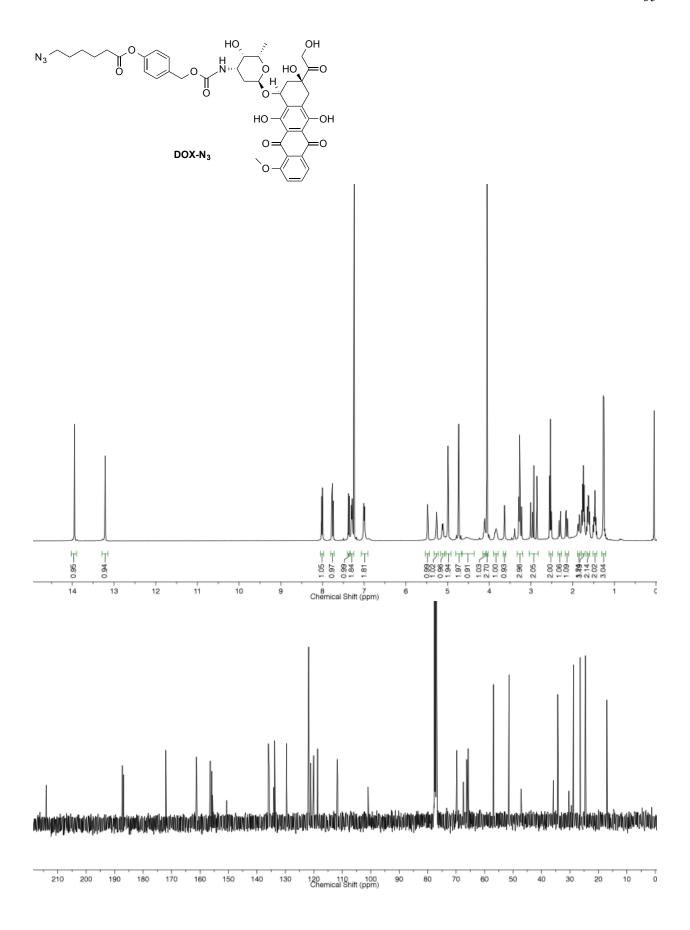


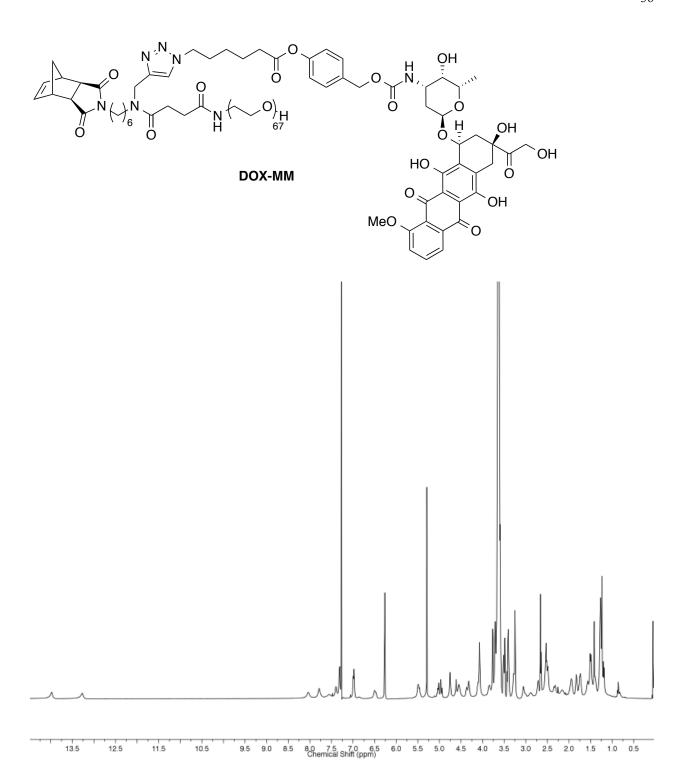
В3

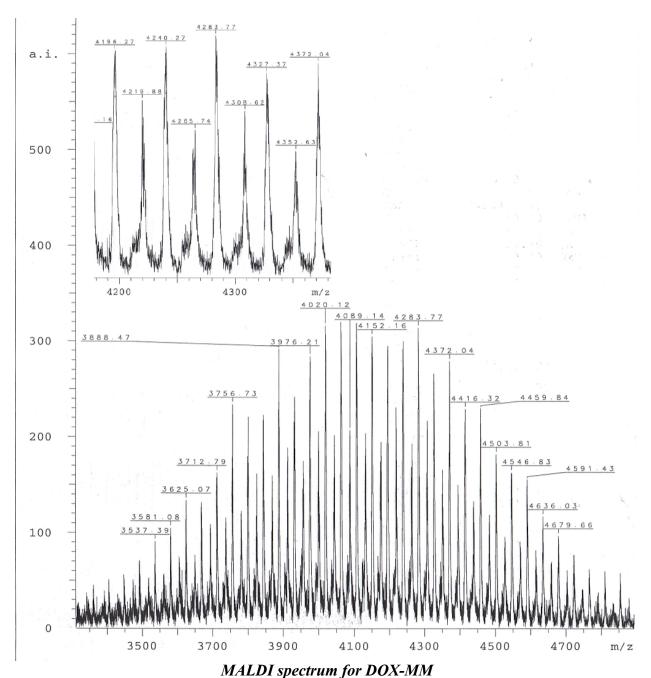












 $(M+H_2O+K)^+$  calcd. for  $C_{199}H_{347}N_7O_{88}K$ : 4282.25, obsd.: 4283.77;  $(M+K)^+$  calcd. for  $C_{199}H_{345}N_7O_{87}K$ : 4264.24, obsd.: 4265.74

## References:

- (1) Love, J. A.; Morgan, J. P.; Trnka, T. M.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 4035–4037.
- (2) Liu, J.; Burts, A. O.; Li, Y.; Zhukhovitskiy, A. V.; Ottaviani, M. F.; Turro, N. J.; Johnson, J. A. J. Am. Chem. Soc. **2012**, *134*, 16337–16344.
- (3) Johnson, J. A.; Lu, Y. Y.; Burts, A. O.; Xia, Y.; Durrell, A. C.; Tirrell, D. A.; Grubbs, R. H. *Macromolecules* **2010**, *43*, 10326–10335.